OBJECTS OF THE PRESENT INVENTION

The main object of the present invention, is to provide antibodies from the egg yolk of hyper immunized hens (HEY antibody) for organochlorine insecticides.

Another object of the present invention is to provide continuos supply of large quantities of consistent, high titer and specific antibodies that can be easily collected and stored.

Yet another object of the present invention is to provide a non-invasive, hence no need to bleed the animal.

Still another object of the present invention, is to provide antibodies that are equally or more sensitive to both polyclonal and monoclonal antibodies produced using mammals.

SUMMARY OF THE PRESENT INVENTION

The present invention provides relates to a process to produce egg yolk antibodies binding to small molecule organochlorine pesticides, by periodic immunization of the poultry birds with a desired hapten-protein conjugate in the breast muscle. Antibodies are harvested from egg yolk after five weeks interval.

DESCRIPTION OF THE INVENTION

Accordingly, the present invention provides a process for the production of egg yolk antibodies binding to small molecule organochlorine pesticides, the said process comprising the steps of:

(a) selecting suitable from poultry birds;

(b) immunizing the poultry birds with known complete adjuvant, each ml of said adjuvant comprising heat killed and dried 1 mg of Mycobacterium tuberculosis (H37Ra, ATCC 25177), 0.85 ml paraffin and 0.15 ml mannide monooleate;

(c) immunizing the birds with 1000 µg conjugate of selected from DDT-OH hapten, Octachloro cyclic hapten, 2,4,5 trichlorophoxyacetic acid β-alanine in breast muscle;

(d) immunizing the birds again with the hapten-protein conjugate as given in step (c) with 500 µg of desired hapten conjugate;

(e) immunizing the birds with hapten-protein conjugate at the intervals of two, three and five weeks;

(f) immunizing the birds thereafter with hapten-protein conjugate at five weeks intervals as long as the bird lays eggs;

(g) harvesting antibodies from the egg yolk of the birds.

An embodiment of the present invention, wherein the desired hapten-protein conjugates having binding properties to DDT, Endosulphan and HCH .

Another embodiment of the present invention, wherein the production of hapten-protein conjugate namely DDT-OH binding to DDT, is as follows:

(a) succinylating 2,2,-Bis(4-chlorophenyl) –1,1,1-trichloroethanol overnight, using excess succinic anhydride in pyridine to obtain N-hydroxy succunimide;

(b) reacting N-hydroxy succinimide 183.5 mg., 0.5mmol in dichloromethane in the presence of dicyclohexylurea and dimethylaminopyridine catalyst in the ratio 1:1:1:1.2 (hapten:NHS:DCC:DMAP) to convert into N-Hydroxy succinimide active ester; and

(c) obtaining active ester of DDT-OH hapten for use in conjugation by isolating dicyclohexylurea and evaporating dichloromethane.

Yet another embodiment of the present invention, wherein the production of hapten-protein conjugate namely octachloro cyclic hapten binding to Endosulphan, is as follows:

(a) dissolving about 3.73 g Heptachlor in 0.1 mol glacial acetic acid by warming;

(b) dissolving 1.085 g Tert-Butyl hypochlorite, in 0.1 mmol glacial acetic acid and adding to the first solution as obtained in step(a);

(c) refluxing the mixture on a water-bath for 1 hour;

(d) separating fine crystals of acetyl-chloro derivative of heptachlor;

(e) washing the crystals with acetone and drying with air;

(f) obtaining the crystalline product in a yield of about 3.02 g, m.p. 238 C. 1.09 g

(g) treating the product to get the pre-hapten 1,3,4,5,6,7,8,8-Octachloro-2-hydroxy-4, 7-methano-3a, and 4,7,7a-tetrahydroindane;

(h) dissolving the pre hapten in dichloromethane by adding N-hydroxysuccinimide and cooling the mixture to 0^0C ;

(i) adding dicyclohexylcarbodiimide followed by dimethylaminopyridine;

(j) stirring the mixture overnight; and

(k) filtering off dichloromethane and evaporating dichloromethane to obtain the active ester of endosulphan.

Still another embodiment of the present invention, wherein the production of conjugate 2,4,5-Trichloro phenoxy acetic acid β- alanine Trichloro benzene (TCB) hapten binding to Hexachloro hexane, is as follows:

(a) adding of ß – alanine spacer arm to 2,4,5 Trichlorophenoxyacetic acid by suspending 10mM, 2.55g of 2,4,5 Trichlorophenoxyacetic acid in 5.95 ml thionyl chloride (9 50 mmol);

(b) refluxing for 1 hour and removing unreacted thionyl chloride by evaporation;

(c) stirring the product with ß – alanine 9 mmol, 0.66g in 7.4 ml of 1M NaOH at 0^0C;

(d) warming the product for over 16 hours at room temperature;

(e) isolating the resulting acid by acidification;

(f) partitioning into ethyl acetate;

(g) washing with water and brine;

(h) giving an yield of crude product hapten containing 2,4,5-Trichlorophenoxyacetic acid (2,4,5-T) as impurity;

(i) dissolving the impurity in acetone to obtain colorless flakes of the Trichlorobenzene(TCB) hapten;